Supplemental Information for *"Meta-analysis of cannabinoid ligand binding affinity and cannabinoid receptor distribution: interspecies differences,"* by McPartland, Glass, Pertwee

Supplementary File, Extended methods

Search strategy

We searched the PubMed-MEDLINE databases for articles published in any language through December 2006, using keywords: affinity, anandamide, cannabinoid, endocannabinoid, tetrahydrocannabinol, tritiated, 2-arachidonoyl glycerol, and 3H (as in "tritiated"). In a previous review article we demonstrated MEDLINE searches yielded only 33% of the available literature (McPartland and Pruitt, 2000), so we adopted an expanded search strategy: Additional affinity data were obtained via the NIMH Psychoactive Drug Screening Program (http://pdsp.cwru.edu/pdsp.php). Gray literature was searched with web engines (www.oaister.org/, www.osti.gov/graylit, www.findarticles.com), *Books in Print* (1984-2006), and by hand searching the reference lists of previous reviews (eg Demuth and Molleman, 2006, Diaz-Laviada and Ruiz-Llorente, 2005, Pertwee, 2005). Retrieved articles were screened for supporting citations, and antecedent sources were retrieved. Finally we contacted world experts and asked them to contribute any studies of which they were aware (see Acknowledgments). We contacted investigators of original studies for clarifications and missing data.

Study selection

Three groups of reviewers independently considered studies for inclusion, and resolved disagreements by consensus. Reviewers were cannabinoid experts, also chosen by their initial willingness to share reprints with the primary author (JM). To be accepted for analysis, an article met the following inclusion criteria:
(1) The study investigated CB_1 or CB_2 in human (*Homo sapiens, Hs*) or rat (*Rattus norvegicus, Rn*). For sensitivity analyses we also included studies of mouse (*Mus musculus, Mm*) and rhesus macaque (*Macaca fascicularis, Mf*). The meta-analysis was limited to normal wildtypes, and excluded data from cannabinoid receptors with point

mutations, cannabinoid receptor knockout mice, or people with neurological diseases. Studies of chimeric constructs were also excluded, such as NG108-15 cells (fusions of *Rn* C6BV-1 and *Mm* N18TG2), F-11 cells (fusions of *Rn* DRG and *Mm* N18TG-2), and hybrid *Rn-Mm* cDNA sequences (eg, GenBank gi:10719923); unfortunately this necessitated the exclusion of classic works by Abood, Howlett, Mackie, Mechoulam, Pertwee, and Sugiura. Studies that used tissues or cells with unidentified receptors were also excluded (eg, fetal lung fibroblasts). Lastly, we excluded data from animals that were previously exposed to cannabinoids (eg, chronic use studies), because receptor affinity and distribution may be affected (data from control animals in those studies, however, were included).

(2) Studies were limited to the nine most-commonly tested cannabinoids: *N*-arachidonoyl ethanolamine (anandamide, AEA), cannabidiol (CBD), cannabinol (CBN), CP55,940, HU210, SR141716A, THC, WIN55,212-2, and *sn*-2 arachidonoyl glycerol (2-AG). Although other cannabinoids have been studied (eg, JWH251, desacetyllevonantradol), they were cited less frequently or in one species only, which made interspecies comparisons difficult.

(3) Studies were limited to two aspects of cannabinoid ligand binding:

A. Receptor-ligand affinity. We included studies that reported affinity as Kd (the concentration of radioligand disassociation) or Ki (the concentration of competitive displacement), measured in nM units. Kd is measured in a saturation assay that determines the concentration of a ligand occupying 50% of receptors at equilibrium. Ki is measured in a competition assay that determines the concentration of a ligand that displaces 50% of a fixed concentration of radioligand; the ligand of interest can be either a cold isotope of the radioligand or a completely different molecule. Kd values should reasonably agree with Ki values for a given ligand, allowing for pipetting inaccuracies and other experimental errors. Fifteen IC_{50} studies were also included after investigators provided information that enabled conversion of IC_{50} to Ki using the Cheng-Prusoff equation. We included data from several studies whose Ki values may be slightly inaccurate, because their Cheng-Prusoff calculations were based upon [^{3}H]CP55940 Kd values obtained from the literature, rather than Kd values measured in their laboratory. These studies cited a Kd of 690 pM (Martin et al., 1999, Pertwee et

al., 2000, Bass et al., 2002, Ligresti et al., 2006), or a Kd of 690 nM (Wiley et al., 1998, Martin et al., 2000, Marquez et al., 2006). Apparently 690 pM was determined in Billy Martin's lab using rat whole brain membranes (B. Martin, personal communication, 2006), but the experiment was not published. Several of the studies cited Compton et al. (1993), who did not report this value; Compton and colleagues used cortical (not whole brain) membranes.

B. CB1 receptor distribution. CB_1 receptors are expressed in unique anatomical distributions, with receptor densities varying amongst brain regions. CB_1 distribution studies were limited to: (i) CB_1 studies of the brain, excluding spinal cord, retina, and the peripheral nervous system. (ii) Studies of adult, healthy animals. Embryological and developmental studies were too few in number to make interspecies comparisons. (iii) Studies that used radioligand binding, either *in situ* autoradiographic techniques or binding to dissected brain regions. Studies that measured CB_1 distribution using immunocytochemistry (via tagged antibodies), or *in situ* hybridization of receptor mRNA (via labeled oligonucleotides) were examined in post-hoc sensitivity studies (described later). Functional radioligand studies were excluded, such as cannabinoid-induced [^{35}S]GTPγS binding and cannabinoid-induced *Fos* expression. Functional studies were excluded because they correlated poorly with autoradiography studies (r = 0.56, (Breivogel et al., 1997)) due to receptor/transducer amplification factors.

Data extraction

We conducted a prospective meta-analysis, where studies were identified and determined to be eligible before the results were synthesized. Reviewers used piloted, standardized data extraction sheets. Supplemental Tables S-1 and S-2 are examples of completed data extraction sheets. For each receptor-ligand affinity study, extracted data included: the species of CB_1 or CB_2 ortholog, ligand Kd and/or Ki, sample variance, sample size, and methodological factors. Methodological factors (covariates) were extracted for later use in subgroup analyses, to test whether they exerted heterogeneous effects upon pooled means. Methodological factors were pre-specified, chosen in advance by *a priori* hypotheses based upon recognized methodological diversity among studies, and *not* undertaken after the results of the studies had been

compiled (*post hoc* analyses). Methodological factors chosen for subgroup analyses must be limited in number, to protect against false positives and data dredging (Thompson and Higgins, 2002). We limited subgroup analyses to five methodological factors, listed below with *a priori* rationales:

1) Affinity assays that used brain sections (eg Herkenham et al., 1990, Thomas et al., 1992), versus the majority of affinity studies that used brain homogenates centrifuged to yield P2 membrane pellets (eg Devane et al., 1988, Compton et al., 1993). Investigators have noted differences in Ki values between brain sections and homogenates, but the differences have never been measured.

2) The use of phenylmethylsulfonyl fluoride (PMSF) or other adjuvants that prevent the breakdown of AEA by catabolic enzymes. Several studies measured the Ki of AEA in brain tissue, with and without PMSF (eg Deutsch and Chin, 1993, Abadji et al., 1994, Childers et al., 1994, Smith et al., 1994). Indeed, the Ki of AEA in brains of FAAH (+/+) mice treated with PMSF (61 nM), nearly equaled the Ki of AEA in brains of FAAH (-/-) mice (52 nM), about 14-fold less than FAAH (+/+) mice without PMSF (Lichtman et al., 2002). However, the use of PMSF reportedly made little difference in spleen tissue (Felder et al., 1995, Lin et al., 1998), or in assays using transfected cells (Felder et al., 1995).

3) Homogenate assays that separated free and bound radioligands by centrifugation (eg Devane et al., 1988, Mechoulam et al., 1995) versus the majority of studies that used rapid filtration (eg Houston et al., 1991, Martin et al., 1991). Disparities in Ki values between these methods have been noted but never assessed.

4) Differences between Kd values and Ki values due to the use of dissimilar radioligands, such as the tritiated agonists [^{3}H]CP55,940, [^{3}H]WIN55212-2, [^{3}H]HU243, and [^{3}H]BAY38-7271. Tritiated inverse agonists, such as [^{3}H]SR141716A, label two populations of receptors (Kearn et al., 1999), yielding results that may differ significantly from those of tritiated agonists (first reported by Thomas et al., 1992). [^{3}H]WIN55212-2 also yields unique displacement curves, possibly due to divergent binding domains (Thomas et al., 2005). A 25-fold disparity has been reported in Ki values obtained from [^{3}H]SR141716A versus [^{3}H]WIN55212-2 (Petitet et al., 1996).

5) Affinity may vary in native tissues *versus* heterologously expressed systems (eg, transfected COS, CHO, and HEK cells). A few investigators have made direct comparisons: Felder et al. (1992) compared the Kd of CP55,940 at *Rn*CB1 in brain homogenates (Kd = 2.3 nM) versus *Rn*CB1-tranfected CHO cells (4.0 nM). Felder et al. (1995) compared the Kd of CP55,940 at *Hs*CB2 in CHO cells (7.37 nM) versus AtT cells (6.94 nM). Rhee et al. (1997) compared the Ki of THC, CBN, and HU210 at *Rn*CB1 in brain homogenates (66.5, 392, 0.1 nM, respectively) versus COS cells (80.3, 211 nM, 0.2 nM). Mauler et al. (2002) compared the Ki of THC at *Hs*CB1 in brain homogenates versus unidentified recombinant cells (13.7 vs 15.3 nM). Thomas et al. (2005) compared the Ki of SR14176A at *Hs*CB1 in cerebellar homogenates versus CHO cells (3.89 vs 4.67 nM). Paugh et al. (2006) compared the Ki of WIN55212-2 at MmCB1 in brain homogenates versus CHO cells (4 vs 11 nM). To wit, some native tissues may express both CB1 and CB2 receptors (Pertwee, 2005). Chin et al. (1999) compared the Kd of WIN55,212-2 at *Hs*CB1 in CHO cells (21.7 nM) versus HEK293 cells (20.4 nM).

Whereas most studies reported one Kd or Ki per ligand, some studies reported ten or more Kd or Ki results, usually from different brain regions. To prevent over-weighting of these studies in the meta analysis, we limited extraction to a maximum of four affinity values per ligand per study. Studies that presented affinity values as negative base-10 logarithms (eg, pKi) were converted to nanomolar (nM) units. If Kd was reported twice from Scatchard analysis and nonlinear regression analysis, we used Scatchard data.

For the meta-analysis of CB1 distribution studies, extracted data included: the species of CB_1, mean density per brain region, and study size. We also extracted two methodological covariates: 1) the radioligand used in the study, and 2) the use of an autoradiographic technique (often measured as optical densities) versus a dissected brain region technique (directly measured as fmol/ml of tissue). In studies that reported non-uniform labeling within a single brain region (eg Glass et al., 1997) divided the hippocampus region into five substrata of CA1, CA2, CA3, and four layers of the subicular complex), we counted the single substrata with the greatest CB1 density per region.

When more than one publication described the results of a single experiment, we extracted data from the publication with the most complete information regarding that experiment. For example, the Ki for THC at *Rn*CB_1 reported by Compton et al. (1993), 40.7 nM, has reappeared in many publications, sometimes rounded to 41 nM (eg Wiley et al., 1998, Mahadevan et al., 2000, Wilcox et al., 1988, Martin et al., 1999). Decisions regarding data extraction and quality assessment met consensus before data was extracted for synthesis.

Quality assessment and publication bias

Dozens of guidelines and checklists for assessing the quality of published studies have been formulated, such as the 22-item CONSORT checklist (Moher and Olkin, 1995). However, the key components in these guidelines, such as patient demographics, blinding of patients and investigators, and dropout rates, and have little relevance in our meta-analysis. Therefore, our guidelines for assessing the quality of studies extracted in this meta-analysis were the inclusion and exclusion criteria. On the other hand, we adapted the guideline checklist by Moher et al. (1999) for quality assessment of our own meta-analytic methodology.

Publication bias describes the tendency to publish positive results in clinical research. This was not considered relevant in signal transduction studies. Publication bias is usually detected with funnel plots, which are scatter plots of effect sizes (Higgins and Green, 2005). Funnel plots could not be constructed from our data, which lacked effect sizes. Rosenthal's fail-safe number, another test for publication bias, is also based upon effect size.

Data synthesis

Data were synthesized quantitatively or qualitatively. Quantitatively, the Ki for each cannabinoid at four receptors (*Hs*CB_1, *Rn*CB_1, *Hs*CB_2, *Rn*CB_2) was synthesized twice, as a pooled mean and as a pooled weighted mean. Because larger studies with less variance should carry more "weight" in a meta-analysis, the weighted mean adjusted a study's mean Ki by the reciprocal of the mean's variance. Thus the weighted affinity (WA) for an individual study is:

$WA = Ki \times 1/(\text{standard error of the mean})^2$

where standard error of the mean (SE) = standard deviation / $\sqrt{n}$,

and where SE = the 95% confidence interval (upper limit – lower limit) / 3.92.

Pooled $WA = \sum(WA) / \sum(1/SE^2)$

Many affinity studies did not provide sample size or sample variance data, making it difficult to calculate a weighted mean. In studies that omitted sample size or sample variance data, it is possible to impute missing data by using the mean sample size and mean variance calculated from studies that did include these data (Piggott, 1994, Higgins and Green, 2005, Wiebe et al., 2006). However, weighted means that use variance imputations must be interpreted with caution if variance is confounded by methologic factors that influence the pooled mean. The validity of a weighted mean depends heavily upon the validity of its underlying linear model theory (Boyce et al., 2005).

To determine whether pooling was statistically appropriate, the coefficient of variation (CV) was determined for each pooled mean. The CV measures data dispersion of a probability distribution, defined as the ratio of the standard deviation to the mean (Reed et al., 2002). CV provides a method of measuring intrinsic variation in a sample or population, because increases in variance caused by increases in means are appropriately adjusted in a common percentage scale. To the CV we applied the Cochrane "skew test" (Higgins and Green, 2005): a skewed mean with excessive heterogeneity was identified by a CV ≥ 1 (standard deviation $\geq$ mean). Medical meta-analyses that measure effects sizes often assess heterogeneity with the Cochran Q test, a form of weighted sums-of-squares. Thus the total heterogeneity (Q_T) among n number of studies is: $Q_T = \sum (1/SE^2)_i (WA_i - \text{pooled } WA)^2$

where $(1/SE^2)_i$ is the inverse variance of the ith study, and WA_i is the weighted mean of the ith study ($i = 1 \ldots n$).

A large Q_T value indicates significant heterogeneity. P values can be obtained by comparing the Q_T with a chi square distribution with $n - 1$ degrees of freedom. This tests whether the weighted means for all of the studies are equal; a significant result indicates the heterogeneity is greater than expected due to sampling error (Higgins and Green, 2005).

Subgroup meta-regression

In the presence of significant heterogeneity, we applied a random effects model (Higgins and Green, 2005), and performed meta-regressions upon subgroups (Thompson and Higgins, 2002). Sugroups were based on methodological covariates, described above ("Data Extraction" section). Meta-regression optimally employs $n \geq 10$ observations per covariant (Higgins and Green, 2005). This proved difficult or impossible for some of the methodological covariates, as noted below:

1) Brain homogenates *versus* brain sections. This comparison utilized CP55940 data at *Rn*CB_1, to optimize sample size (i.e., the ligand and receptor that provided the greatest number of observations per covariant).

2) PMSF *versus* no PMSF in brain tissue utilized AEA data at *Rn*CB_1. The effects of PMSF upon 2-AG affinity could not be analyzed because of insufficient data. Ditto PMSF effects upon 2-AG affinity in brain or spleen.

3) PMSF *versus* no PMSF in CB_1-transfected cells utilized AEA data at *Hs*CB1, although $n < 10$.

4) PMSF *versus* no PMSF in CB_2-transfected cells utilized AEA data at *Hs*CB2, although $n < 10$.

5) Filtration *versus* centrifugation comparisons utilized CP55940 data at *Rn*CB_1, although a paucity of centrifugation data necessitated pooling of THC and HU210 data with the CP55940 data at *Rn*CB_1 for the centrifugation covariate. Pooling was performed by normalizing THC and HU210 data; we used preliminary pooled means to make algebraic recalculations. For example, the preliminary pooled means of CP55940 and THC at *Rn*CB_1 were 0.92 nM and 45.3 nM, respectively. Thus THC 46 nM at *Rn*CB_1 (Devane et al., 1992) was normalized [46 x (0.92/45.3) = 0.934], and pooled with CP55940 data.

6) Kd *versus* Ki comparisons utilized CP55940 data at *Rn*CB_1.

7) Ki measured by different radioligands utilized CP55940 data at *Rn*CB_1, which provided ample samples of Ki measured by [^{3}H]CP55,940 and [^{3}H]SR141716A. Paucity of data for [^{3}H]WIN55212-2 and [^{3}H]HU243 was resolved by pooling of

WIN55212-2 data (at *Rn*CB_1) with CP55940 data, after appropriate normalization using preliminary pooled means, as described above.

8) The comparison of native tissues *versus* transfected cells utilized CP55940 data at *Hs*CB_1. A scarcity of native tissue data was remedied by pooling of THC and WIN55212-2 data (at *Hs*CB_1), after appropriate normalization.

Final synthesis of affinity and distribution data

Meta-regression identified several methodological aspects that contributed to heterogeneity. Based on these results, we withdrew the following heterogeneous data: affinity data reported in studies that used sectioned brain tissue, AEA data in studies that used homogenized brain tissue not treated with PMSF, and Ki data in studies that used [^{3}H]SR141716A as radioligand. To these adjusted means we reapplied the CV-skew test. Persistently skewed means were submitted to Grubb's test, using an outlier calculator (GraphPad, www.graphpad.com/quickcalcs/Grubbs1.cfm). Studies with data outliers were inspected for methological flaws, and in some cases were removed from synthesis, as noted in Table S-1 and Table S-2. Notably, outliers were frequently reported as logarithmic transformations in the original literature, which is a common method of dealing with skewed data. This treatment of pooled means with large SDs is also known as "moderator analysis" and reduces artifact when calculating pooled means (Glass et al., 1981). The CV-skew test sometimes could not be met for sample sizes of $n \leq 3$.

Comparisons of means were tested for statistical significance with the Mann-Whitney U or Wilcoxon rank sum test, performed with SYSTAT 5.2.1 (Systat, Inc., Evanston, IL, USA). Although our data fit normal distribution 'rule of thumb' skew and kurtosis values (in the range +2 to -2), some data did not meet normality assumptions when subjected to a formal goodness-of-fit test (Lilliefors correction of Kolmogrov-Smirnov test), therefore we used non-parametric tests.

If we re-analyzed data with parametric ANOVA, only one statistical inference changed in Table 1-3: the Ki of AEA at *Hs*CB_1 and *R*nCB_1 became significantly different ($p = 0.004$).

For CB_1 distribution data, the studies presented in Supplemental Table S-6 were synthesized qualitatively as scalar transformations or as narrative comparisons. Scalar transformations were performed upon every study that reported CB_1 density in ≥2 brain regions. The relative density of each brain region was ranked (arrayed) from highest value to lowest value. Rank orders from individual studies were then aggregated using a bubble-sort algorithm (Sese and Morishita, 2001). Additionally, we used narrative comparisons to describe aspects of CB_1 distribution that were not quantified in original studies (eg, receptor patterns in autoradiography images).

Sensitivity analysis

A series of analyses were then performed to judge *a priori* hypotheses and the robustness of the results. We began by identifying additional methodological covariates based upon post-hoc observations. Next, the validity and numerical precision of our pooled Ki and Kd values were tested, using three approaches:

1) The pooled Ki and Kd values were scalar transposed into rank orders, and ranked from highest affinity to lowest affinity. These results were compared to rank orders derived by a method independent of arithmetic means. For every original study that examined ≥2 ligands per receptor, ligands were ranked from highest to lowest affinity; individual studies were then aggregated using a bubble-sort algorithm. Affinity rank order derived from the bubble-sort algorithm was then compared to a rank order derived from the pooled means.

2) Pooled Ki and Kd values for *Hs* and *Rn* were compared to the few studies that made direct interspecies comparisons. 3) Pooled Ki and Kd values for *Rn* were compared with pooled Ki and Kd means from *Mm* studies. *Rn* and *Mm* cross-species differences no doubt exist, but the rodents share similarity on a molecular level – *Rn*CB_1 and *Mm*CB_1 are 99.8% identical (diverging at one residue out of 473 amino acids), compared to *Rn*CB_1 and *Hs*CB_1 differing at 12 residues plus a codon deletion (McPartland et al., 2006). *Rn*CB_2 and *Mm*CB_2 are 93.3% identical (McPartland et al., 2006).

Sensitivity analyses for CB_1 brain distribution data also utilized cross-species comparisons. Regional rank orders for *Rn*CB_1 were examined for consistency with

MmCB_1 rank orders, and *Hs*CB_1 rank orders were examined for consistency with two other primates, rhesus macaque (*Macaca fascicularis, Mf*) and baboon (*Papio hamadryas, Ph*).

Abadji, V., Lin, S., Taha, G., Griffin, G., Stevenson, L. A., Pertwee, R. G. and Makriyannis, A. (1994). (R)-methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. *J Med Chem,* **37,** 1889-93.

Bass, C. E., Griffin, G., Grier, M., Mahadevan, A., Razdan, R. K. and Martin, B. R. (2002). SR-141716A-induced stimulation of locomotor activity. A structure-activity relationship study. *Pharmacol Biochem Behav,* **74,** 31-40.

Boyce, M. S., Irwin, L. L. and Barker, R. (2005). Deomographic meta-analysis: synthesizing vital rates for spotted owls. *Journal of Applied Ecology,* **42,** 38-49.

Breivogel, C. S., Sim, L. J. and Childers, S. R. (1997). Regional differences in cannabinoid receptor/G-protein coupling in rat brain. *J Pharmacol Exp Ther,* **282,** 1632-42.

Childers, S. R., Sexton, T. and Roy, M. B. (1994). Effects of anandamide on cannabinoid receptors in rat brain membranes. *Biochem Pharmacol,* **47,** 711-5.

Chin, C. N., Murphy, J. W., Huffman, J. W. and Kendall, D. A. (1999). The third transmembrane helix of the cannabinoid receptor plays a role in the selectivity of aminoalkylindoles for CB2, peripheral cannabinoid receptor. *J Pharmacol Exp Ther,* **291,** 837-44.

Compton, D. R., Rice, K. C., De Costa, B. R., Razdan, R. K., Melvin, L. S., Johnson, M. R. and Martin, B. R. (1993). Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. *J Pharmacol Exp Ther,* **265,** 218-26.

Demuth, D. G. and Molleman, A. (2006). Cannabinoid signalling. *Life Sci,* **78,** 549-63.

Deutsch, D. G. and Chin, S. A. (1993). Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol,* **46,** 791-6.

Devane, W. A., Dysarz, F. A., 3rd, Johnson, M. R., Melvin, L. S. and Howlett, A. C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol,* **34,** 605-13.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science,* **258,** 1946-9.

Diaz-Laviada, I. and Ruiz-Llorente, L. (2005). Signal transduction activated by cannabinoid receptors. *Mini Rev Med Chem,* **5,** 619-30.

Felder, C. C., Joyce, K. E., Briley, E. M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A. L. and Mitchell, R. L. (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol Pharmacol,* **48,** 443-50.

Felder, C. C., Veluz, J. S., Williams, H. L., Briley, E. M. and Matsuda, L. A. (1992). Cannabinoid agonists stimulate both receptor- and non-receptor-mediated signal transduction pathways in cells transfected with and expressing cannabinoid receptor clones. *Mol Pharmacol,* **42,** 838-45.

Glass, G. V., McGaw, B. and Smith, M. L. (1981) *Meta-analysis in social research,* SAGE Publications, Beverly Hills, CA.

Glass, M., Dragunow, M. and Faull, R. L. (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience,* **77,** 299-318.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., de Costa, B. R. and Rice, K. C. (1990). Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A,* **87,** 1932-6.

Higgins, J. P. T. and Green, S. (2005). Cochrane Handbook for Systematic Reviews of Interventions 4.2.5 [updated May 2005]. *In: The Cochrane Library. Chichester, UK: John Wiley & Sons, Ltd*.

Houston, D. B., Evans, D. M., Howlett, A. C. and Melvin, L. S. (1991). 3H]CP55940 binding to the cannabinoid receptor. *Dupont Biotech Update,* **6,** 21-27.

Kearn, C. S., Greenberg, M. J., DiCamelli, R., Kurzawa, K. and Hillard, C. J. (1999). Relationships between ligand affinities for the cerebellar cannabinoid receptor CB1 and the induction of GDP/GTP exchange. *J Neurochem,* **72,** 2379-87.

Lichtman, A. H., Hawkins, E. G., Griffin, G. and Cravatt, B. F. (2002). Pharmacological activity of Fatty Acid amides is regulated, but not mediated, by Fatty Acid amide hydrolase in vivo. *J Pharmacol Exp Ther,* **302,** 73-9.

Ligresti, A., Cascio, M. G., Pryce, G., Kulasegram, S., Beletskaya, I., De Petrocellis, L., Saha, B., Mahadevan, A., Visintin, C., Wiley, J. L., Baker, D., Martin, B. R., Razdan, R. K. and Di Marzo, V. (2006). New potent and selective inhibitors of anandamide reuptake with antispastic activity in a mouse model of multiple sclerosis. *British Journal of Pharmacology,* **147,** 83-91.

Lin, S., Khanolkar, A. D., Fan, P., Goutopoulos, A., Qin, C., Papahadjis, D. and Makriyannis, A. (1998). Novel analogues of arachidonylethanolamide (anandamide): affinities for the CB1 and CB2 cannabinoid receptors and metabolic stability. *J Med Chem,* **41,** 5353-61.

Mahadevan, A., Siegel, C., Martin, B. R., Abood, M. E., Beletskaya, I. and Razdan, R. K. (2000). Novel cannabinol probes for CB1 and CB2 cannabinoid receptors. *J Med Chem,* **43,** 3778-85.

Marquez, N., De Petrocellis, L., Caballero, F. J., Macho, A., Schiano-Moriello, A., Minassi, A., Appendino, G., Munoz, E. and Di Marzo, V. (2006). Iodinated N-acylvanillamines: potential "multiple-target" anti-inflammatory agents acting via the inhibition of t-cell activation and antagonism at vanilloid TRPV1 channels. *Mol Pharmacol,* **69,** 1373-82.

Martin, B. R., Beletskaya, I., Patrick, G., Jefferson, R., Winckler, R., Deutsch, D. G., Di Marzo, V., Dasse, O., Mahadevan, A. and Razdan, R. K. (2000). Cannabinoid properties of methylfluorophosphonate analogs. *J Pharmacol Exp Ther,* **294,** 1209-18.

Martin, B. R., Compton, D. R., Thomas, B. F., Prescott, W. R., Little, P. J., Razdan, R. K., Johnson, M. R., Melvin, L. S., Mechoulam, R. and Ward, S. J. (1991). Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. *Pharmacol Biochem Behav,* **40,** 471-8.

Martin, B. R., Jefferson, R., Winckler, R., Wiley, J. L., Huffman, J. W., Crocker, P. J., Saha, B. and Razdan, R. K. (1999). Manipulation of the tetrahydrocannabinol side chain delineates agonists, partial agonists, and antagonists. *J Pharmacol Exp Ther,* **290,** 1065-79.

Mauler, F., Mittendorf, J., Horvath, E. and De Vry, J. (2002). Characterization of the diarylether sulfonylester (-)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. *J Pharmacol Exp Ther,* **302,** 359-68.

McPartland, J. M., Matias, I., Di Marzo, V. and Glass, M. (2006). Evolutionary origins of the endocannabinoid system. *Gene,* **370,** 64-74.

McPartland, J. M. and Pruitt, P. L. (2000). Benign prostatic hyperplasia treated with saw palmetto: a literature search and an experimental case study. *J Am Osteopath Assoc,* **100,** 89-96.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., Gopher, A., Almog, S., Martin, B. R., Compton, D. R. and et al. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol,* **50,** 83-90.

Moher, D., Cook, D. J., Eastwood, S., Olkin, I., Rennie, D. and Stroup, D. F. (1999). Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. *Lancet,* **354,** 1896-900.

Moher, D. and Olkin, I. (1995). Meta-analysis of randomized controlled trials. A concern for standards. *Jama,* **274,** 1962-4.

Paugh, S. W., Cassidy, M. P., He, H., Milstien, S., Sim-Selley, L. J., Spiegel, S. and Selley, D. E. (2006). Sphingosine and Its Analog, the Immunosuppressant 2-Amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol, Interact with the CB1 Cannabinoid Receptor. *Mol Pharmacol,* **70,** 41-50.

Pertwee, R. G. (2005). Pharmacological actions of cannabinoids. *Handb Exp Pharmacol***,** 1-51.

Pertwee, R. G., Gibson, T. M., Stevenson, L. A., Ross, R. A., Banner, W. K., Saha, B., Razdan, R. K. and Martin, B. R. (2000). O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties. *Br J Pharmacol,* **129,** 1577-84.

Petitet, F., Marin, L. and Doble, A. (1996). Biochemical and pharmacological characterization of cannabinoid binding sites using [3H]SR141716A. *Neuroreport,* **7,** 789-92.

Piggott, T. D. (1994) In *The Handbook of Research Synthesis*(Eds, Cooper, H. and Hedge, L. V.) Russell Sage Foundation, New York, pp. 163-174.

Rhee, M. H., Vogel, Z., Barg, J., Bayewitch, M., Levy, R., Hanus, L., Breuer, A. and Mechoulam, R. (1997). Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. *J Med Chem,* **40,** 3228-33.

Sese, J. and Morishita, S. (2001). Rank aggregation method for biological databases. *Genome Informatics,* **12,** 506-507.

Smith, P. B., Compton, D. R., Welch, S. P., Razdan, R. K., Mechoulam, R. and Martin, B. R. (1994). The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *J Pharmacol Exp Ther,* **270,** 219-27.

Thomas, B. F., Francisco, M. E., Seltzman, H. H., Thomas, J. B., Fix, S. E., Schulz, A. K., Gilliam, A. F., Pertwee, R. G. and Stevenson, L. A. (2005). Synthesis of long-chain amide analogs of the cannabinoid CB1 receptor antagonist N-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyra zole-3-carboxamide (SR141716) with unique binding selectivities and pharmacological activities. *Bioorg Med Chem,* **13,** 5463-74.

Thomas, B. F., Wei, X. and Martin, B. R. (1992). Characterization and autoradiographic localization of the cannabinoid binding site in rat brain using [3H]11-OH-delta 9-THC-DMH. *J Pharmacol Exp Ther,* **263,** 1383-90.

Thompson, S. G. and Higgins, J. P. (2002). How should meta-regression analyses be undertaken and interpreted? *Stat Med,* **21,** 1559-73.

Wiebe, N., Vandermeer, B., Platt, R. W., Klassen, T. P., Moher, D. and Barrowman, N. J. (2006). A systematic review identifies a lack of standardization in methods for handling missing variance data. *J Clin Epidemiol,* **59,** 342-53.

Wilcox, A. J., Weinberg, C. R., O'Connor, J. F., Baird, D. D., Schlatterer, J. P., Canfield, R. E., Armstrong, E. G. and Nisula, B. C. (1988). Incidence of early loss of pregnancy. *N Engl J Med,* **319,** 189-94.

Wiley, J. L., Compton, D. R., Dai, D., Lainton, J. A., Phillips, M., Huffman, J. W. and Martin, B. R. (1998). Structure-activity relationships of indole- and pyrrole-derived cannabinoids. *J Pharmacol Exp Ther,* **285,** 995-1004.